The Panama Canal Species

OF THE

Genus Anopheles

JAMES ZETEK, B. A.,
Entomologist, Ancon, C. Z.

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This paper is intended primarily for the use of our district sanitary inspectors, district physicians, post surgeons, and others connected with or interested in our sanitation problems. The general bionomics of the members of this genus will be considered in a future report.

PART 1.—TAXONOMY.

General Characteristics.

The *Anopheles* constitute the group "anophelines" of the tribe Culicini of the dipterous family *Culicidæ*. They are among the most generalized and lowest of all mosquitoes. The palpi are long in both sexes, as long as, or longer, than the proboscis. This proboscis is slender and straight. In the male the palpi terminate in a distinct club, and the antennæ are densely plumose. The scutellum is rounded and not trilobed as in most of the Culicini. The wings are usually spotted. The first tarsal joint of the hind legs is longer than the tibia; some systematists have referred to this long first joint as the "metatarsus," but this term is a misnomer and its use has caused no few confusions in the past.

As yet we have no satisfactory characters with which to separate our various species of *Anopheles* in the pupal stage. It is difficult often to tell some of the pupæ from those of other mosquitoes.

The larvæ as a rule rest in a horizontal position, against the surface film. They are able to maintain this position on account of the palmate hairs or tufts present on the abdominal segments. These hairs form rosettes which spread out more or less fan like, against the surface film and thus help support the larva. The number of these palmate hairs and the shape of the individual hairs are of taxonomic value. These rosettes are easily seen when the larva is at rest against the surface film, appearing as small black indentures in this film. A low-power hand lens (10x)

is very useful in determining the number and disposition of these tufts. The air tube is absent. On the eighth abdominal segment are two chitinous plates, the lower margins of which are provided with a series of irregular teeth, the size, number, and arrangement of which may prove to be of value in separating the various species. Color and spots on larvæ and pupæ mean nothing. This can be easily demonstrated by securing a large series of larvæ from a single locality and allowing them to pupate and then recovering the adults that emerge.

Anopheles eggs are very distinct and different from those of other mosquitoes. They are laid singly, or in small numbers, and almost always form beautiful geometric patterns on the surface of the water. Each egg is provided with a peculiar hydrostatic organ or "float" which looks like a white hemisphere on each side of the egg. Its purpose is to keep the eggs afloat. As yet nothing has been seen which would separate our *Anopheles* eggs to species.

A few technical terms are unavoidable. They are, in fact, a big necessity in order to be accurate and clear in our descriptions of the structures present. It is not advisable nor sound practice to dispense with such terms and substitute for them terms used in common, everyday parlance. To do so creates much confusion. The reader will find it necessary to know these few technical terms because if any of the papers on mosquitoes are read, these terms are used, and if not understood by him, the subject matter is not grasped. To facilitate the understanding of what these different terms represent, two plates were prepared with the important structures labeled. (Plates E and F.)

It should be remembered that the males of many genera of mosquitoes besides *Anopheles* have long palpi, and that some mosquitoes other than *Anopheles* have spotted wings, so that it becomes very important to have a very clear picture in the mind as to just what the *Anopheles* resemble, so that at a glance, almost, it is possible to say whether or not the specimen at hand is an *Anopheles*. I have made no reference to the peculiar scales present but those interested more deeply in this subject should consult the works of Theobald, of Giles, and of Howard, Dyar, and Knab.

Many larvæ also assume a horizontal position against the surface film, and at times look very much like *Anopheles;* the presence of the palmate hairs, and the absence of a breathing tube are sufficient to make the determination. The head of the *Anopheles* larvæ is also distinctly elongate, and rotates very easily at

the neck. Most confusion on the Canal Zone has been due to mistaking *Uranotænia* larvæ for *Anopheles* larvæ. At first glance there is much resemblance in these two genera, but *Uranotænia* has a breathing tube, and on its head it has stout spines.

The Panama Canal Zone Species of Anopheles.

The valid species now known from the Canal Zone are:

A. (*Cellia*) *argyritarsis* Robineau-Desvoidy, 1827.
A. (*Cellia*) *albimanus* Wiedemann, 1821.
A. (*Cellia*) *albimanus* var. *tarsimaculata* Goeldi, 1905.
A. (*Anopheles*) *pseudopunctipennis* Theobald, 1901.
A. (*Anopheles*) *eiseni* Coquillett, 1902.
A. (*Arribalzagia*) *punctimacula* Dyar and Knab, 1906.
A. (*Arribalzagia*) *apicimacula* Dyar and Knab, 1906.
A. (*Dendropædium*) *neivai* Dyar and Knab, 1917.
A. (*Dendropædium*) *hylephilus* Dyar and Knab, 1917.

Certain other names have been applied in the past to our *Anopheles* which later studies have shown to be synonymous with names already in use. The following list gives the names that are no longer tenable, and in parentheses are given the valid equivalents. This list only has reference to names used in the literature bearing on our mosquito fauna.

A. gorgasi Dyar and Knab (is *tarsimaculata*).
A. albipes Theobald (is *albimanus*).
A. albimana Wiedemann (is *albimanus*).
A. cruzii Dyar and Knab, in part (is *neivai*).
A. lutzii Theobald, not Cruz, (is *neivai*).
A. franciscanus McCracken (is *pseudopunctipennis*).
A. niveopalpis Ludlow (is *eiseni*).
A. malefactor Dyar and Knab (is *punctimacula*).
A. punctipennis Truby (is *pseudopunctipennis*).
A. strigimacula Ludlow (is *punctimacula*).

The *Anopheles punctipennis* is a misprint which occurs on page 21 of Col. Albert E. Truby's report of the Health Department for the year 1917. The *A. strigimacula* (Ludlow, 1914, p. 64) is in all probabilities *punctimacula*, and perhaps *apicimacula* is also involved; true *strigimacula* D. & K. inhabits tropical Mexico. In *strigimacula* the light scales on the legs are yellow, not white; I consider the Canal Zone record by Doctor Ludlow a misidentification.

Doctor Darling (1910, p. 5) grouped the commoner Canal Zone *Anopheles* into three fairly well defined classes: (1) The white-footed species, such as *albimanus*, *tarsimaculata*, and *argyritarsis*;

(2) those with uniformly dark legs, such as *pseudopunctipennis* and *eiseni*; (3) those with speckled legs, such as *apicimacula* and *punctimacula*. There remains a fourth group to be added, namely, (4) *bromelicolous Anopheles*, i. e., those species that live in the water held by the leaves of Spanish Bayonets, Heliconias, epiphytic Bromelias, etc., represented on the isthmus by *neivai* and *hylephilus*.

Artificial Key to Adults and Larvæ.

In preparing a key to our species, I kept in mind the fact that it must be as simple as possible, and yet contain the most conspicuous characters which definitely "mark" the species. Such a key works best when the specimens are in good condition. Mosquitoes which have been rubbed during handling and therefore have many scales gone, or which lack portions of their legs, palpi, etc., are somewhat difficult to place accurately. Therefore it is suggested that the descriptions given in Howard, Dyar, and Knab, and in the Insecutor Inscitiæ Menstruus (*see* list of references) be read carefully, with good specimens at hand, so that other characters as well may be noted.

After some work is done on the identification of our *Anopheles* it will become very evident that the better the specimens, the easier and more certain will be the results. And perhaps, at least such is the hope, this revelation will be productive of greater efforts to have specimens in as good a condition as is possible, to take good care of the material, especially if it is to be sent to the entomologist for identification.

In the following key the hind legs only are referred to. The middle broad white band on the palpi of *tarsimaculata* is often represented by only a few white scales, and from such a condition every degree of gradation is obtained until the typical white band is had:

First tarsal joint entirely black.
- Tarsi 2 to 5 entirely black.
  - Tibia entirely black ..... *pseudopunctipennis* Theob.
  - Apical one-quarter of tibia yellowish-white ..... *eiseni* Coq.
- Tarsi 2 to 5 mostly white.
  - Last tarsal joint entirely white ..... *argyritarsis* Rob.-Desv.
  - Basal one-third of last joint black.
    - Palpus with tip white, followed by a broad black band, then a narrow white band ..... *albimanus* Wiede.
    - Palpus with tip white, followed by a narrow black band, then a broad white band, a narrow black one, then a narrow white band ..... *albimanus* var. *tarsimaculata* Goeldi

First tarsal joint with more or less white.
Hind legs distinctly speckled.
Wing with three conspicuous black spots; last tarsal joint white, *punctimacula* D. & K.
Wing like *punctimacula* but with an additional distinctly black spot at apex; last tarsal joint black except at extreme base and apex, *apicimacula* D. & K.
Hind legs not speckled. Femora and tibia streaked with white; tarsi white apically.
Wing very dark, with four costal bars..... *hylephilus* D. & K.
Basal two-thirds of costal portion of wing black, followed by two small white spots separated by a large black one..... *neivai* D. & K.

Of the above species, the following are the commonest: *Albimanus, tarsimaculata,* and *pseudopunctipennis;* then come *punctimacula* and *apicimacula* which are abundant locally at times. *Argyritarsis* and *eiseni* are rather rare, while *hylephilus* and *neivai* will be gotten only after a patient and careful search in the jungle.

A key to the larvæ is not very simple because it requires the use of a low-power microscope, and what the sanitary inspector most needs is some key which he can make use of in the field, with perhaps only a 10x hand magnifier. The rule has been established in the department that the presence of *Anopheles* larvæ or pupæ within a sanitated district calls for immediate attention; therefore, from a practical standpoint, the field identification is not necessary to species. Specimens can always be taken and upon arrival at the office may be identified to species. In the following key to larvæ only the full-grown stage is considered. Younger stages should be allowed to mature, and it is a good policy to also allow some to reach the adult stage.

Palmate hairs on segments 1 to 7, inclusive, of abdomen, those on the first segment usually smaller .....*albimanus* and *tarsimaculata.*
Palmate hairs on segments 2 to 7, inclusive, of abdomen.
Elements of palmate hairs slender and smoothly pointed.....*argyritarsis.*
Elements of palmate hairs strap-like, tips truncate.....*neivai.*
Elements of palmate hairs broad to three-fifths from base, then by means of notches come to a tip.....*eiseni.*
Palmate hairs on segments 3 to 7, inclusive, of abdomen.
A single smooth hair near base of antenna.....*pseudopunctipennis.*
A tuft at basal third of antenna.....*punctimacula* and *apicimacula.*
(The larva of *hylephilus* is unknown.)

Notes on our Species of Anopheles.

The detailed descriptions given in Vol. IV. of Howard, Dyar, and Knab, and in certain of the references to Lasecutor Inscitiæ Menstruus, should be con-

sulted. Citations to Theobald and to Giles are not given because the descriptions in these occur in Howard, Dyar, and Knab; this 4-volume monograph will remain a standard work of reference on mosquitoes for decades to come.

ANOPHELES (CELLIA) ALBIMANUS Wiedemann.

A. albimanus Wiedemann, Dipt. Exot., 10, 1821.
A. cubensis Agramonte, El Progreso Medico, x. 460, 1900.
A. argyritarsis albipes Theobald, Mon. Culic., i, 125, 1901.
A. albimanus Busck, Smith. Misc. Colls., quart. iss., lii, 57, 1908.
A. albimanus Darling, Stud. Rel. Malaria, I. C. C., 6, 1910.
A. albimanus Howard, Dyar, and Knab, Mosq. N. & C. Am. & W. I., iv, 979, 1917.
A. albimanus Dyar, Ins. Insc. Mens., vi, 151, 1918.

The characters given in the key to adults clearly separate this common species from its congeners. It often happens, however, when specimens are hard and dry, that the last tarsal joint is broken off, in which case a hurried glance might confuse such a specimen with *argyritarsis*. Therefore it is good policy always to make sure that the tarsal joints are complete before identifying a specimen as *argyritarsis*. A good character upon which *argyritarsis* can be separated from *albimanus* and *tarsimaculata* is the color of the light scales of the wing; they are white in *argyritarsis* and yellow in the others. The mesonotum of this white-footed group of *Anopheles* is grayish brown in color and has three velvet-black spots on it, these forming an isosceles triangle, with the apex posterior. The broad black band following the white tip, on the palpus, has no white scales; if any such scales are present, the specimen should be referred to the variety *tarsimaculata*.

The larvæ have palmate hairs on segments 1 to 7, inclusive, of the abdomen, those on the first segment often being very small and situated nearer the median line than are the others. The elements of these palmate hairs are slender, straight, and pointed; sometimes they are broader than is shown in the drawing. In the field these palmate tufts are readily seen as small black indentations in the surface film, and by counting them, identifications to groups can be made readily, though determination to species is a much more difficult matter. Floating the larvæ in milk makes these tufts stand out clearer.

Albimanus and *tarsimaculata* are inseparable in the larval stage. The color of these larvæ ranges from dark brown or almost black to lighter shades, and to bright green, often mottled, depending

largely on the character of the food and habitat. Some writers claim there are distinctive marks or spots on which the species can be separated in the field. I have seen nothing as yet that was of value in this direction; spots and color in *Anopheles* larvæ have very little, if any, diagnostic values. All sorts of intergradations can be found in individuals from the same lot.

The lateral plates on the eighth abdominal segment have a series of long and short spines alternating irregularly. It is quite possible the number, size, and disposition of these spines may prove to be constant characters that may help in determining larvæ to species; as yet too little data is at hand to make any deductions.

Tropical America, Greater Antilles, and southern Florida. It is our dominant *Anopheles*, breeds the year round, though most abundant during the rainy season.

ANOPHELES (CELLIA) ALBIMANUS Wiede. *Var. TARSIMACULATA* Goeldi.

A. tarsimaculata Goeldi, Os. Mosq. no Para, 133, 1905.
A. gorgasi Dyar and Knab, Journ. N. Y. Ent. Soc., xv, 198, 1907.
A. tarsimaculata Busck, Smith. Misc. Colls., quart. iss., lii, 58, 1908.
A. tarsimaculata and *gorgasi* Darling, Stud. Rel. Malaria, I. C. C., 6, 1910.
A. tarsimaculata Howard, Dyar, and Knab, Mosq. N. & C. Am. & W. I., iv, 975, 1917.
A. tarsimaculata Dyar, Ins. Insc. Mens., vi, 151, 1918.

In my remarks under *albimanus* I referred already to this species. The big difference, and only one, between it and that species, is the broad white band on the palpus, thus making three white bands, whereas *albimanus* has but two.. But this middle white band is very often represented by only a few white scales, and from such a condition, by more and more white, until the typical *tarsimaculata* type is obtained. All such variations may occur in a single lot of pupæ, taken from the same place, and what is of greater significance, true *albimanus* is also obtained from this same lot. This fact establishes the point that *tarsimaculata* can not be considered a distinct species, that at most it is only a variety of *albimanus*. Knab (1912) in a footnote on page 198 referred to *tarsimaculata* as a geographic race of *albimanus*, but since then it has always been considered a distinct species.

Dyar (1918, p. 151) refers to a similar intergradation in specimens he received from Guayaquil, Ecuador, sent him by Mr. F. Campos R. My first reference to this condition was made in 1915 (*see* page 24).

Considered from a practical standpoint, especially with respect to their pathogenicity, they are equally bad and it makes very little difference whether they are separated into two forms. There is, however, a preference for brackish water by *tarsimaculata*, while *albimanus* will live in both fresh and brackish water. This fact is based upon our statistics of mosquitoes examined, and it should be noted that most of our catches came from the low, swampy regions about Gatun and from there to the Atlantic Coast.

Howard, Dyar, and Knab (1917, iv., p. 978) state "it is only from Panama northward that *tarsimaculata* and *albimanus* occur together, the latter species extending southward from Panama only on the western side of the Andes." Tropical American mainland (Nicaragua to Peru), Lesser Antilles. Next to *albimanus* it is our commonest *Anopheles*.

ANOPHELES (CELLIA) ARGYRITARSIS Rob.-Desv.

A. argyritarsis Robineau-Desvoidy, Mém. Soc. d'Hist. Nat., iii, 411, 1827.
A. argyritarsis Busck, Smith. Misc. Colls., quart. iss., lii, 59, 1908.
A. argyritarsis Darling, Stud. Rel. Malaria, I. C. C., 5, 1910.
A. argyritarsis Howard, Dyar, and Knab, Mosq. N. & C. Am. & W. I., iv, 967, 1917.
A. argyritarsis Dyar, Ins. Insc. Mens., vi, 150, 1918.

There are two big distinguishing characters which mark off this species: (1) The last tarsal joint is entirely white, and (2) the light colored scales on the wings are white in color, not yellow. The wings usually have more black scales than do those of *albimanus* and *tarsimaculata*, but there is great variation in this feature.

The larvæ have palmate hairs only on segments 2 to 7, inclusive, but the first segment should always be looked at with care because in both *albimanus and tarsimaculata* the palmate hairs on this segment are usually smaller and are set further inward than the others, and hence are easy to overlook, especially so if the specimen is at all dark colored and dense. The elements of these palmate tufts are not quite as slender as in the other two species, but they resemble them very much. The degree of thickness of these hairs varies considerably in the individuals of the same lot. The lateral plates on the eighth segment have a series of five or six long spines separated from each other by two or three short irregular intervening ones.

Tropical American mainland and the Lesser Antilles. It is not common in the catch from quarters, but the larvæ are frequently

found during inspections. They are often found in containers hidden among dense vegetation. There appears to be no decided seasonal variation in their abundance.

ANOPHELES (ANOPHELES) PSEUDOPUNCTIPENNIS Theob.

A. pseudopunctipennis Theobald, Mon. Culic., ii, 305, 1901.

A. franciscanus McCracken, Ent. News, xv, 12, 1904.

A. pseudopunctipennis Busck, Smith, Misc. Colls., quart. iss., lii, 57, 1908.

A. pseudopunctipennis and *franciscanus* Darling, Stud. Rel. Malaria, I. C. C., 7, 8, 1910.

A. pseudopunctipennis Howard, Dyar, and Knab, Mosq. N. & C. Am. & W. I. iv., 1015, 1917.

A. pseudopunctipennis Dyar, Ins. Insc. Mens., vi, 143, 1918.

This is perhaps the most majestic of our *Anopheles;* it is the largest of all. Its legs are very long, brownish black in color throughout, and all three pairs rest on the same surface. The very tips of the femora and tibia are narrowly whitish in color. The mesonotum is dark brown along its sides, with the center portion a very broad stripe, ashy gray in color. This stripe stands out clearly to the naked eye, and is enough to identify the species.

The larvæ are also easy to recognize. They have palmate hairs only on segments 3 to 7, inclusive. The elements of these tufts are broad to about their middle, and then rapidly narrower till they form a blunt point; occasionally there are a few indentations. There is no tuft on the basal third of the antennæ, which absence marks off this species from the *punctimacula* group. The larvæ are usually large, with the thorax prominent, and since this species prefers waters rich in algal growth, they are usually green in color.

Tropical American and adjacent warmer regions (Dyar), southern United States to Peru and Argentine. A widely distributed species, commonest in Panama during the dry season. It does not enter houses readily, unless these are unscreened.

ANOPHELES (ANOPHELES) EISENI Coq.

A. eiseni Coquillett, Journ. N. Y. Ento. Soc., x, 192, 1902.

A. tibiamaculata Neiva, Brazil-Medico, xx, 288, 1906.

A. eiseni Busck, Smith. Misc. Colls., quart. iss., lii, 58, 1908.

A. eiseni Darling, Stud. Rel. Malaria, I. C. C., 10, 1910.

A. eiseni Howard, Dyar, and Knab, Mosq. N. & C. Am. & W. I., iv, 1003, 1917.

A. eiseni Dyar, Ins. Insc. Mens., vi, 143, 1918.

A. niveopalpis Ludlow, Psyche, xxvi, 166, 1919.

This is one of the rarer species of *Anopheles*, and is sylvan in character. It is quite small, compared with the preceding species. There are two very conspicuous characters which distinguish it. The wing appears to be smoky in color, without any spots excepting two white ones, of which the one at the tip of the wing is the most prominent. These spots are usually a dirty white in color. The hind tibiæ are peculiarly marked, there being a yellowish white area which involves the apical fourth of these tibiæ; this light bar is often referred to as a "white knee."

Doctor Ludlow (1919) described *niveopalpis* from a specimen she received from Mr. James B. Shropshire, sanitary inspector of the United States Army stationed on the Canal Zone, and collected by him at the Camacho Reservoir, Empire. She placed the specimen tentatively in the subgenus *Stethomyia*. From the description of this new species I was unable to differentiate it from *eiseni* and hence asked Dr. Harrison G. Dyar to kindly examine the type specimen. Doctor Dyar did so, confirming our opinion that *niveopalpis* is *eiseni*.

The larva is not often found in routine inspections, and usually not near habitations. It has a dainty appearance, usually light brown in color, and without spottings. It is about equally wide throughout, widest at the thorax. It has six pairs of palmate hairs, segments 2 to 7, inclusive. The elements of these tufts are equally wide to about three-fourths of the way; thence, by a distinct constriction, or a few serrations, these elements come to a blunt point. The antennæ have a few fine spines on their inner margins, and a hair tuft very close to the base. These larvæ breed usually in pot holes in streams, and in tree holes.

Tropical America (Dyar): Mexico to Panama, Brazil, Trinidad, W. I.

ANOPHELES (ARRIBALZAGIA) PUNCTIMACULA D. & K.

A. punctimacula Dyar and Knab, Proc. Biol. Soc. Wash., xix, 136, 1906.

A. malefactor Dyar and Knab, Journ. N. Y. Ento. Soc., xv, 198, 1907.

A. malefactor Busck, Smith. Misc. Colls., quart. iss., lii, 59, 1908.

A. malefactor and *punctimacula* Darling, Stud. Rel. Malaria, I. C. C., 9, 1910.

A. malefactor Howard, Dyar, and Knab, Mosq. N. & C. Am. & W. I., iv, 1000, 1917.

A. punctimacula Dyar, Ins. Insc. Mens., vi. 147, 1918.

It is unfortunate that the old name *malefactor* which has been used for so many years must now be sunk into synonomy and

the name *punctimacula* used instead. But this procedure is entirely proper since the two names represent only one species and *punctimacula* is the oldest of the two, hence the valid name. The unique specimen collected by W. M. Black at Colon on February 2, 1904, and named *punctimacula* by Dyar and Knab, was, on Mr. Knab's responsibility, placed as a synonym of *apicimacula* in the excellent monograph by Howard, Dyar, and Knab. Later (1918) Dyar reexamined the type specimen of *punctimacula* and discovered it was not *apicimacula*, but really *malefactor*. This last change in nomenclature is in all probabilities a final one.

The *punctimacula* group of our Anophelenes are the prettiest and most handsome of all, and from Doctor Darling's studies (1910) the safest as well, for they do not transmit malaria. They are readily distinguished by their speckled legs, large wings, and the very distinct black spots on these wings. In addition to the characters given in the keys and sketches, the following one may sometimes be of value: The first tarsal joint of the front legs has six white rings, the second joint has three, the third and fourth have narrow white rings at both basal and apical ends, and the fifth joint is entirely white.

The larva has a distinct tuft of hairs at the basal third of the antennæ, and five pairs of palmate hairs (segments 3 to 7, inclusive). The elements of these palmate hairs resemble those of *eiseni* but begin to draw out to a blunt point a little beyond the middle. The lateral plates on the eighth segment have a series of very long and very short spines, alternating irregularly. The antennal tuft is very conspicuous and should always be looked for in all larvæ having but five pairs of palmate hairs.

Panama only; not very abundant, preferring shaded habitats.

ANOPHELES (ARRIBALZAGIA) APICIMACULA D. & K.

A. apicimacula Dyar and Knab, Proc. Biol. Soc. Wash., xix, 136, 1906.
A. apicimacula Busck, Smith. Misc. Colls., quart. iss., lii, 59, 1908.
A. apicimacula Darling, Stud. Rel. Malaria, I. C. C., 10, 1910.
A. apicimacula Howard, Dyar, and Knab, Mosq. N. & C. Am. & W. I., iv, 995, 1917.
A. apicimacula Dyar, Ins. Insc. Mens., vi, 148, 1918.

The most important difference between this species and the preceding one is the presence of a distinctly black conspicuous spot at the tip of the wing, thus making four main spots along the costal border. The leg ornamentation is also different, though it should be remembered that such spottings as occur on the legs

are often apt to vary. The sketches show the hind leg ornamentation. The first tarsal joint of the front legs has three white spots, the second, third, and fourth have a basal white ring, the second has a narrow white ring at the middle, the last joint is pale at the tip. This front leg ornamentation differs considerably from that of *punctimacula*. Sometimes the last tarsal joint of the hind legs is entirely white.

The larva is not well known; it resembles greatly *punctimacula* and I doubt if it can be satisfactorily separated from it.

Mexico, Central America, and Trinidad (Dyar); rare.

ANOPHELES (DENDROPAEDIUM) NEIVAI H. D. & K.

A. lutzii Busck (not Cruz, not Theobald), Smith, Misc. Colls. quart. iss., lii, 58, 1908.

A. cruzii Dyar and Knab (in part), Proc. U. S. Nat. Mus., xxxv, 53, 1908.

A. cruzii Darling (not Dyar and Knab), Stud. Rel. Malaria, I. C. C., 10, 1910.

A. sp., near *cruzii* Jennings, Journ. Ec. Ento., v, 135, 1912.

A. neivai Howard, Dyar, and Knab, Mosq. N. & C. Am. & W. I., iv, 986, 1917.

A. neivai Dyar, Ins. Insc. Mens., vi, 146, 1918.

This, and the following species, are bromelicolous *Anopheles*, i. e., their larvae live in the scant water held between the leaves, sheaths, etc., of bromelias, heliconias, and such plants, especially epiphytes. They are sylvan in character, and consequently rare.

The main distinguishing characters in the adult are the two white spots involving the apical third of the costa of the wing, and the peculiarly streaked legs. The tarsi of the hind legs resemble very much those of *hylephilus* excepting that the black bands are two to three times as broad.

The larva has six pairs of palmate hairs; these are small, and their elements are strap like, with truncate tips. The lateral plates on the eighth abdominal segment have the teeth uniform and long.

Panama and Costa Rica, probably also Mexico (Dyar).

ANOPHELES (DENDROPAEDIUM) HYLEPHILUS D. & K.

A. hylephilus Dyar and Knab, Ins. Insc. Mens., v, 38, 1917.

A. hylephilus Dyar and Knab, Ins. Insc. Mens., vi, 146, 1918.

Very similar to the preceding species, differing mainly in the wing coloration. There are four white spots involving the costal margin. The hind tarsi have more white than those of *neivai*. The larva is unknown.

No doubt other bromelicolous *Anopheles* are present in our region, hence efforts should be made to collect larvæ in tree holes, epiphytic bromelias, heliconias, Spanish bayonets, etc.

Manoa, Venezuela; Guayaquil, Ecuador; Gatun, C. Z. Only a single female from each locality.

PART 2.—COLLECTION, STUDY, AND CARE OF MATERIAL.

It is not intended in this section to discuss all the methods in vogue, but merely those that have been used with success here, or which, in my opinion, would be well to adopt. It is believed that uniformity in the methods used will systematize the work and yield better results.

Methods of Collection.

Larvæ and pupæ.—No general rules can be given as to where best to search for larvæ and pupæ. A sane guide is to overlook no body of water, and especially to search for small pockets of water that are protected by tall grass. The only exception would be water that has been recently oiled or larvacided. As far as possible, the collector should aim to learn as much as he can about the environment and the species inhabiting it. This demands close observation in the field, the taking of copious notes, and accurate determinations of the larvæ collected. This study of the ecology of the mosquito larvæ is, after all, the most complete understanding one can get both of the insects and of their habitats. It is a study of relations, of responses, of constant adjustments to the peculiar conditions in the complete environment. The wide-awake, alert inspector, who aims to learn and correlate these facts, is the inspector who usually gets the best results. He knows his district and he knows the intricacies of the problems he must face and solve each day.

A precaution that is not often heeded and which often will yield negative results needs to be stated. It is that when a shadow passes over the surface of the water, the larvæ as a rule go down to the bottom and remain there several minutes. This is a general statement which will need some modification according to the species concerned, but it holds good for most *Anopheles* larvæ. This positive response to shadows accounts for the fact why sometimes breeding is not located. If no larvæ are obtained in the first dishful of the water, it is well to make several more trials before being certain none are present.

The most necessary apparatus for collecting mosquito larvæ and pupæ includes a small, white plate, a good pipette, a supply of clean glass receptacles, a suitable carrying case, and a field notebook. If crabholes, tree holes, or similar habitats are to be explored, it will be necessary to have in addition a large rubber bulb fitted to a long glass tube.

The important thing about all collections is not to mix up the different lots of larvæ and pupæ. It is well to have blank labels pasted to the glass receptacles, and as soon as a collection is made from a given place, to enter the data concerning same in the field notebook, and to mark on the label the distinguishing number or letter. If inspections are of daily occurrence, then the label should contain also the date, and if several men are out making collections, then the initials of the collector should be added. Too much care can not be taken, and if a practice is made of keeping accurate notes, no extra work or hardship will be noticeable.

For all general purposes the plate should be three or four inches in diameter, not too shallow, and it should be unbreakable. Sometimes a 6-inch plate is very useful. Some collectors prefer a white dipper. The plate method, however, brings the inspector closer to the surface of the water, and this is important. In using the plate, it should be thrust into the water diagonally so as to intercept any larvæ which may attempt to reach bottom after the initial scare.

The pipette is an instrument which deserves attention, because a poor one causes endless annoyance. The ordinary medicinal dropper is usually unsatisfactory, yet, if a better one is not available, it may serve fairly well the purpose, provided the tip is broken off so as to give an opening about 3 mm. in diameter. This tip should be glazed in a flame so that the larvæ or pupæ passing through it will not be injured. A more satisfactory pipette can be made as follows: Glass tubing $\frac{3}{8}$ inch bore is used; one end is drawn out so that the bore will be at least 3 mm. in diameter, or better still, oval in shape, the long axis 4 mm. and the short one 3 mm. About one inch from this tip a 30-degree bend is made. The rest of the tube should be about 4 inches long. The small tip should be well glazed. A large, heavy, rubber medicine-dropper bulb should be used if available; if not, a piece of tight-fitting soft rubber tubing about $1\frac{1}{2}$ inches long may be used by plugging up the free end with a good piece of cork.

Proper receptacles for the larvæ and pupæ are very important, and yet, usually this matter receives the least attention. Uniformity in size is an advantage. Perhaps the best type of container is the small-size bottle used for grape juice, which holds about 4 ounces of water. Neckless shell vials, 4 inches by 1 inch are also suitable. Each receptacle should be clean, provided with a blank label, and should have the tip covered with a piece of gauze. About 1 inch of water in each container is enough. Care should be taken to protect the specimens from too much jarring en route, and from exposure to intense light and heat rays. In transferring the larvæ from plate to tube, care should be taken not to include predaceous insect larvæ.

A suitable carrying case is very essential. This may be in the form of a box made of light wood, or it may be a bag made out of canvas and containing pockets in which the bottles and other equipment may fit. A good case gives maximum protection to the material collected, and also provides against loss en route. Trouser or coat pockets are not satisfactory places for mosquito larvæ tubes.

The field notebook should be of pocket size, and should have the name and address of the owner clearly written on it so that if lost and found, it can be returned to him. Notes taken in the field should be as full as possible so that the habitat can be properly described. Very often a detail which appears to be insignificant is found to be very important when the various strands of data are woven together.

In collecting larvæ from crabholes, tree holes, etc., a large rubber bulb with a long glass tube is necessary in order to reach down into the scant water which often exists in such holes. The water thus sucked up is emptied into the plate and the larvæ present transferred to a clean container, together with some of the water in which found. If there is not enough water in the tree hole, it will be necessary to add clean water, but in doing so, care should be taken so that no larvæ are accidentally introduced. All collections from tree holes must be kept separate. For larvæ in the water held between leaves of bromelias, heliconias, etc., the small pipette is usually sufficient.

When it is desired to collect *Anopheles* larvæ in great numbers, to supply special purposes for the Board of Health Laboratory, a different procedure is in order. A pail, covered with cloth, makes a good container. A net, made of fine mesh bolting cloth, about 6 inches in diameter, makes a useful collecting apparatus. It

is used where the larvæ are abundant, and, as the water drips out of the net, the larvæ remain inside. The net is then inverted over the plate or the pail, and by means of a pipette, clean water is poured over it, so as to dislodge the larvæ and pupæ. Or, if water is already in the pail, it may be enough to simply wet the inverted net in this water. I prefer to first dislodge the larvæ into a plate because then I can eliminate any predacious animals that may be present.

Adults.—The first rule to remember with respect to adult mosquitoes is that they should be handled as little as possible. They are frail insects, and the scales rub off very easily. It is therefore advisable to caution the colored mosquito catchers not to even touch the mosquitoes with their fingers, and to expect of these catchers only specimens, and not a detailed list of the number of *Anopheles*, *Culex*, etc. The mosquito catcher is supposed to collect mosquitoes; he is not a student in entomology, nor an aspirant for the post of sanitary inspector.

Where and how to collect depends entirely upon circumstances and problems. Buildings that are well screened, and used as barracks, may have attached to them special traps, such as the C. H. Bath type, described in Le Prince and Orenstein, 1916, page 214. The mosquitoes so caught are usually killed by means of burning sulphur, the various traps being placed in a tight box. Exposure to these sulphur-dioxide fumes should be short, only enough to kill the insects. Each trap should be numbered so that the catch may be known from every location. These traps should be indicated exactly on a map of the town, and the catch from each trap compared with respect to wind direction, location of nearest breeding areas, etc.

The usual collection of adults in the houses is described fully by Le Prince and Orenstein, pages 211-213. The killing tube is a neckless shell vial, 4 inches by 1 inch, in the bottom of which is placed a plug of cut rubber bands, about 1 inch high; this is covered with a small disk of cotton, and over this is a tight-fitting circle of white blotting paper. The cork must be good quality and fit tight. The rubber plug is saturated with chloroform, and only enough should be used to saturate the rubber. The tube must always be maintained dry. The heat of the hand holding the killing tube liberates enough chloroform to kill the insect. The reason we use chloroform is because it kills very rapidly.

In transferring mosquitoes from the tube to the pill box, the fingers must not be used. The flies should be allowed to slide gently down the tube into the pill box. A little care and patience will enable the collector to empty his catch without in any way damaging the specimens, and to distribute it evenly in the pill box without the use of a pencil or pin. So much good material is made worthless and practically unidentifiable just because the colored mosquito catcher does not exercise enough care, and if sanitary inspectors will give this matter more attention, it is believed the mosquito catchers will gladly learn and follow the better technique. The catch should not be emptied where there is much wind.

The pill box method of keeping adults is perhaps the most satisfactory. Only the large size pill box should be used (4.5 cm. diameter). Upon leaving the office the mosquito catcher should be provided with enough of these pill boxes, already prepared, so that he will be able to take care of his catch promptly. Mosquitoes should not be left in the killing tubes for any great length of time, only sufficiently to kill them, but care should be taken that the insects are dead before transferring them to the pill boxes.

Pill boxes are prepared as follows: In the bottom is placed a drop or two of creosote, or creosote and naphthalene (saturated mixture), or a saturated solution of naphthalene in chloroform; the creosote-naphthalene preparation is best. This is put in to prevent book lice (psocids) from entering and eating up the specimens. Then a circular disk of cotton batting is placed against the bottom. If cotton batting is unavailable, ordinary cotton may be used provided it is smooth. The mosquito catch is carefully allowed to slide into the pill box. Then another disk of cotton batting is placed gently against the mosquitoes, the cover applied, and the data written thereon. The mosquitoes must *not* be packed in tightly. The object of the cotton covering is to prevent the specimens from moving about while the boxes are in transit. Not more than 25 or 30 mosquitoes should be put into a single large pill box. If more than that number were caught, then additional pill boxes should be used, labeling each box of the same series consecutively.

The following data should be placed on each pill box: (1) Name of station, (2) number or name of quarters where specimens were collected, (3) date collection was made, (4) whether trap or hand catch, (5) initials of the collector. If the catch repre-

sents the whole town, and is not differentiated according to buildings, then No. 2 is not necessary. Should the catch represent unusual conditions, such as outdoor night collecting, that fact should also be stated. Boxes should be labeled in the field, at the time the specimens have been placed in them. It is also advisable to have a collecting bag or hand box in which the collector may carry his outfit.

The best time for collecting indoors is at or just after dawn, and at or just after dusk. During the rest of the day the mosquitoes are well secluded and hard to find. During the hours indicated they are nearly all at the screens.

Since mosquito catching is a very important measure in the reduction of malaria, too much care can not be taken in the selection of good mosquito catchers, and enough additional men should be trained from time to time so that if more catchers are required, or if any are sick, that there will always be others ready to step into the work. The eyes of all mosquito catchers should be examined, both regarding sight and color perception.

Sometimes mosquito catchers become lazy or otherwise indisposed to do their work honestly and well. Such men sometimes breed out mosquitoes in order to have a supply on which to draw, or else, they retain mosquitoes which the sanitary inspector already examined, to re-present them at another day. Extremely dry specimens turned in by a mosquito catcher always should create a suspicion, and if, in the case of *Anopheles*, there are males present in the catch, this too, should require investigation. The best procedure in each case is to make believe nothing out of the ordinary was noted, and to quietly keep tab of the guilty collector's acts.

Another source of valuable material and information is collecting in the field at and after dusk. Often this requires only quiet and patience, because if *Anopheles* are present, they will soon locate the bait. Two men are better than one because one of them can collect from the back of the other. Tents and mosquito bar nets are valuable additions to this sort of collecting (*see* Le Prince and Orenstein, 1916, and Zetek, 1915). Mosquitoes so caught should be kept separated according to 10-minute periods, so as to determine period of maximum abundance. The pill boxes should be carefully labeled as to date, time, and exact locality; the field notes taken should include data regarding direction and velocity of the wind, character of the weather, description of the locality, and such other data as has any bearing upon the catch.

If the sanitary inspector does not determine the material caught in his district, and wishes it sent to the entomologist for identification, then he should not handle the catch at all, so that it will reach its destination with as little damage as possible.

Care and Study of Material at the Office.

Our sanitary inspectors have no laboratory, hence I refer to their *sanctum sanctorum* as an office. The most important thing to always remember is to bring in the material in as good shape as possible. For larvæ and pupæ this involves protection against intense light and heat and as little shaking as possible. For adults it means as little handling as possible with fingers, dry killing tubes, and properly prepared pill boxes. The more important references which take up the care and study of mosquitoes are indicated with an asterisk (*see* last section).

A portion of the office should be set aside for these immature stages, and as soon as any batch is no longer of value, it should be removed so that the place will not accumulate unnecessary things. Ordinary white soup plates make good receptacles for developing larvæ. As soon as pupæ are formed, they should be taken out and placed in neckless shell vials, 4 inches by 1 inch, or in 4-ounce grape-juice type bottles, these covered with gauze so that the adults that emerge shall not escape into the room. For the general technique regarding care and aeration of the larvæ, see Darling, 1910, and Zetek, 1912.

It is important that the soup plates, tubes, etc., all be properly labeled so that the various lots and stages shall not become mixed. For this purpose a ruled book is useful, and it should have several ruled columns with the following headings: Number, date, locality, name, and remarks. The number should be consecutive, and should be preceded by a letter which will separate the several sanitary districts. If several species are present in a single lot, then each may be designated by the letters *a*, *b*, *c*, etc., added to the number. These numbers should appear on all material of that same series.

When adults have emerged in the tubes, they should be left for a period of about one hour until they have reached their full coloration. They should then be removed and killed. The following type of tube is very useful for this purpose: A neckless shell vial, 4 inches by 1 inch, is taken and the bottom broken out. Then two or three thicknesses of gauze are stretched over the broken end, and fastened to the tube by means of adhesive

tape. This tube permits the adults to enter it with greater ease, especially if it is tipped toward the light and the other tube or bottle kept in the dark. When the adults have entered this tube, it is placed on a piece of clean blotting paper, and a few drops of ether or chloroform are dropped on the gauze top.

Since great care must be taken in handling adult mosquitoes, only very fine pointed forceps should be used, or better still, a horsehair loop cemented in a small-bore glass tube. This loop may be made as follows: A 6-inch piece of very narrow-bore glass tube is taken and filled with any good adhesive. I use liquid celluloid. Then a piece of horsehair is bent and the free ends thrust into the tube. The loop should not be over 1 cm. long and about 3 mm. wide. This tube may be used as it is, or it may be inserted into a larger-bore tube in order to give a better hold.

In use, the loop is carefully inserted through the legs so that the mosquito is caught in it. It can then be handled as freely as is necessary, provided no wind is present, and examined with a hand lens to determine the species. Then it can be either mounted, or placed in a pill box.

To examine larvæ for determination to species, it is first necessary to kill a few. A white porcelain casserole is the best for this purpose. Water is added to it and brought to a boil; then by means of a pipette, as many of the larvæ as may be desired, are dropped into this hot water. This kills them at once, and the larvæ are in a straight position. They may now be examined under the microscope. Only low power is necessary. For rapid work it is not even important to use a cover glass. The dead larvæ are picked up with the pipette and dropped on a glass slide, and this placed under the microscope. All the important structures necessary for identification will be easily seen. If, however, difficulty is experienced, then a cover glass may be used, and the specimen viewed under a higher power.

The horsehair loop is a very good instrument with which to transfer larvæ from one dish to another, i. e., when permanent mounts are made, but as it is not my purpose to dwell on the making of permanent mounts, I shall not go into this subject any further.

It is well to preserve in alcohol determined *Anopheles* larvæ. Two dram vials, 70 per cent alcohol, and good corks are necessary. After the larvæ are killed in the hot water, they may be transferred direct into the alcohol. The data should be written on a

good quality white paper, with a soft black pencil, and placed inside of the vial. The cork should, after everything is complete, be capped with paraffin to prevent the rapid evaporation of the alcohol.

Adults may be kept stored without much trouble, provided a few simple measures are followed. First of all, a suitable container must be had. A well-fitted wooden box is satisfactory. A large glass museum jar, either cylindrical or rectangular, with a tight-fitting glass top, is much better. It is assumed that the pill boxes have been treated as already explained, and hence if care was taken in handling the adults, these should be in good condition when stored in the jar. Inasmuch as the disinfectant used disappears with time, and hence the specimens may become infested with psocids, it is well to fumigate the jar of pill boxes about once a month. This fumigation is very simple; all that is necessary is to pour some carbon-bisulphide into the jar, preferably upon a small dish placed on top of the pill boxes, and to have the lid closed tightly. The dosage is about three pounds of carbon-bisulphide to 1,000 cubic feet of space, and the specimens should remain in fumigation at least 24 hours.

Protection from mold is also necessary. This is usually done by keeping the jar in a dry closet or near some artificial heat. If this is done, and the lid is always kept on, very little trouble from mold will result.

By exercising a little care and observing the few details mentioned, no difficulty should be encountered; the better quality of the material so obtained, the ease with which it can be studied, and the wealth of information that is obtained, warrant every effort in this direction.

Transportation of Material Collected.

I have already considered the transportation of material collected in the field to the office of the district sanitary inspector. Briefly summarized, the various stages of the mosquitoes must be considered as delicate organisms, easily killed or damaged, and hence must be handled with much care, be protected against direct heat and intense light, be jarred as little as possible, and as regards the adults, not to be handled with the fingers, and to be caught in a dry killing tube and placed in a properly prepared pill box.

The transportation of mosquitoes to the Board of Health Laboratory or elsewhere is another matter. Each locality must look into its transportation facilities and make arrangements which will facilitate the safe transportation of larvæ and pupæ. On the Canal Zone we use the hospital car which is attached to certain Panama Railroad trains. It is always best to send the larvæ and pupæ in some sort of a glass bottle, preferably of wide mouth, such as the jars used for jam; but the ordinary type used for beer, catsup, etc., are very serviceable. Not more than one or two inches of water should be present, and the mouth must be securely protected by gauze. Protection against jarring and intense light is necessary. For this purpose they should be sent in a wooden box, in the bottom of which, and all around the bottles, some excelsior, waste, or other similar substance is packed. The keeper of the car should permit no one to handle the specimens en route.

Larvæ and pupæ to be sent to entomologists living at long distances, where it would take over a few hours to reach, or where it is impracticable to ship the larvæ alive, should be sent preserved in 70 per cent alcohol, the vials well sealed with good cork and capped with paraffin. The usual type of mailing tube is satisfactory. Full data should accompany the specimens, especially the name and address of the sender.

Adults may be sent in the prepared pill boxes, provided they are packed in a good mailing tube, or in a small wooden box. Otherwise they are very apt to arrive crushed and useless.

The Keeping of Records.

Lack of enough data is the usual drawback to full and correct interpretation of results. If notes are not taken right in the field, when the observations are made, they are usually not taken, or if the inspector waits until he arrives at the office, he is usually tired and hence not in the best frame of mind, or else the facts are no longer fresh in the mind. The time to take notes is right in the field, and if a few contractions and signs are used, much may be written with very little effort. These field notes, to be of maximum value, should embrace all of the physical, biotic, and historic factors present in the environment. It means a careful description of the composition and dynamics of the complete habitat, augmented by accurate determinations of the species present. It is a scientific study productive of just that kind of

definite knowledge of the breeding habits and places, behavior, and general interrelationships of our various species of mosquitoes which is necessary in determining the measures and efforts that must be taken in the reduction of these pests. Generalizations based upon theoretical conclusions as to where larvæ should or might perhaps be, usually mean very little, and are usually costly as well as only partially effective.

To those who have not made it a practice to take field notes, the idea proposed may appear to demand too much of their time, and perhaps to some it may even appear a waste of time. This, however, is not an innovation; it is the application of a well-known and tried-out principle, accepted in all scientific work.

These field notes should be kept in permanent form at the station office, properly cross-indexed so that they may be available at any moment. The exact system to use may be left to the inspector at each station, but whatever method is adopted, the aim should be for flexibility and ease for future expansion.

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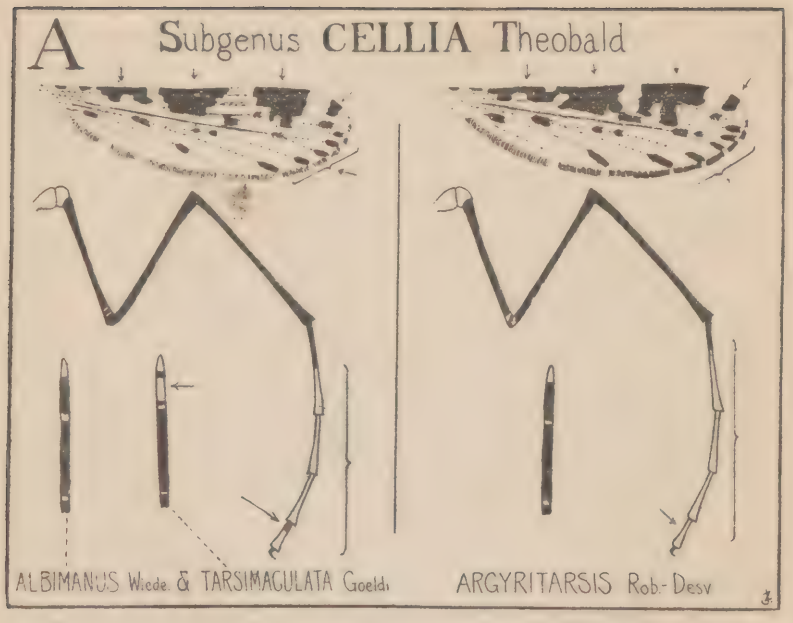
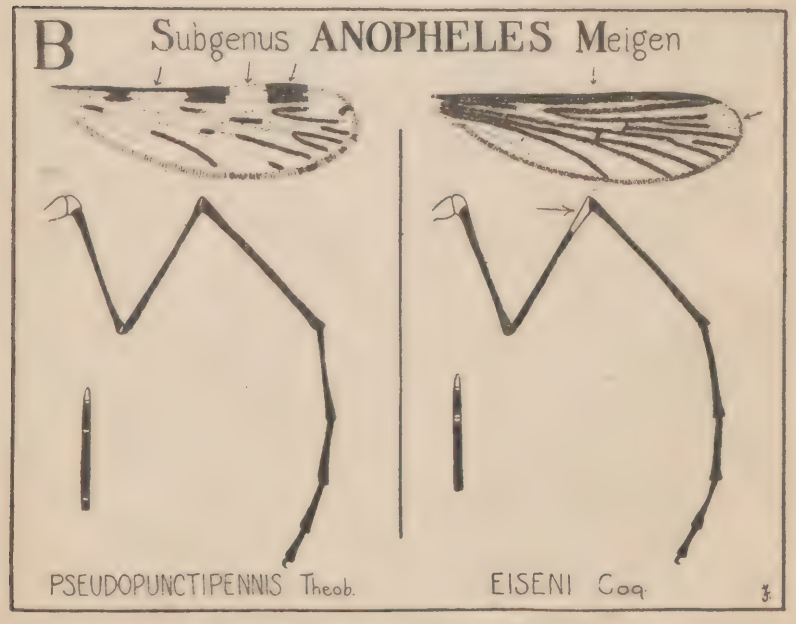

A
Subgenus CELLIA Theobald
ALBIMANUS Wiede. & TARSIMACULATA Goeldi
ARGYRITARSIS Rob.-Desv
♂


B
Subgenus ANOPHELES Meigen
PSEUDOPUNCTIPENNIS Theob.
EISENI Coq.
♂


C Subgenus ARRIBALZAGIA Theobald
PUNCTIMACULA Dyar & Knab
APICIMACULA Dyar & Knab

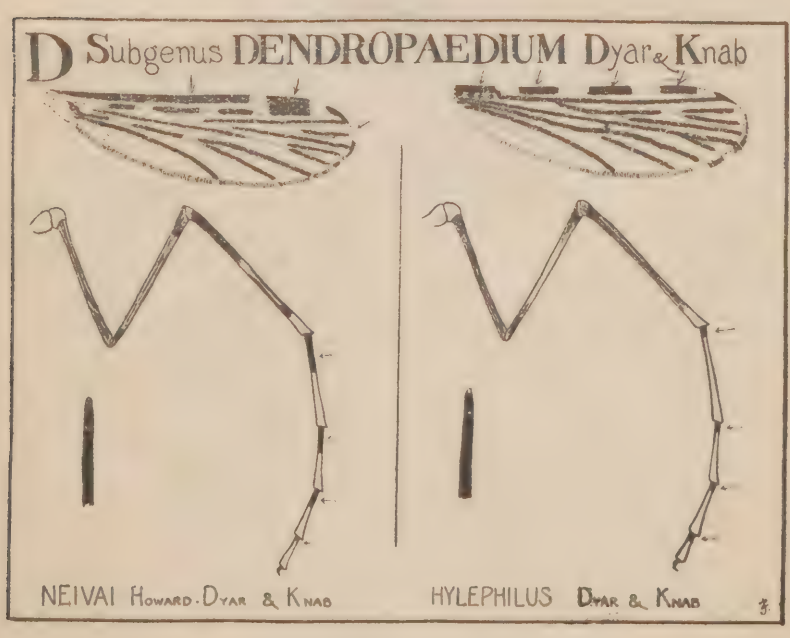

D Subgenus DENDROPAEDIUM Dyar & Knab
NEIVAI Howard-Dyar & Knab
HYLEPHILUS Dyar & Knab

PLATE E.

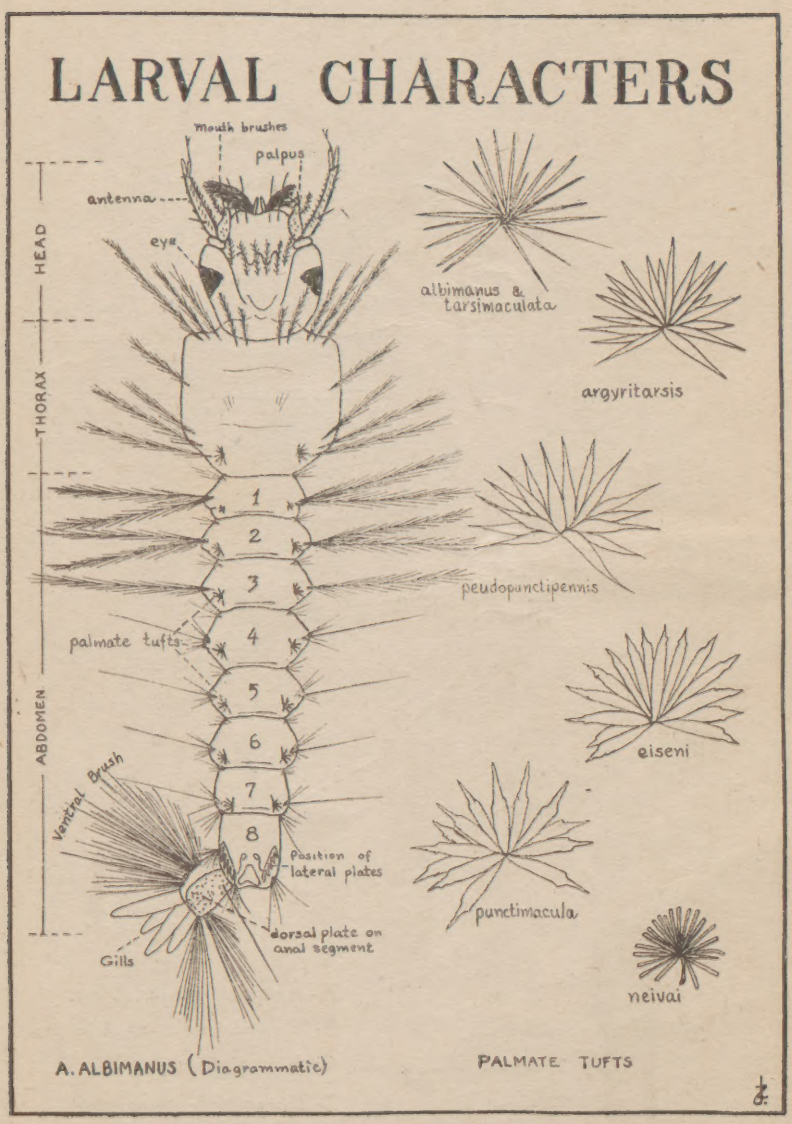
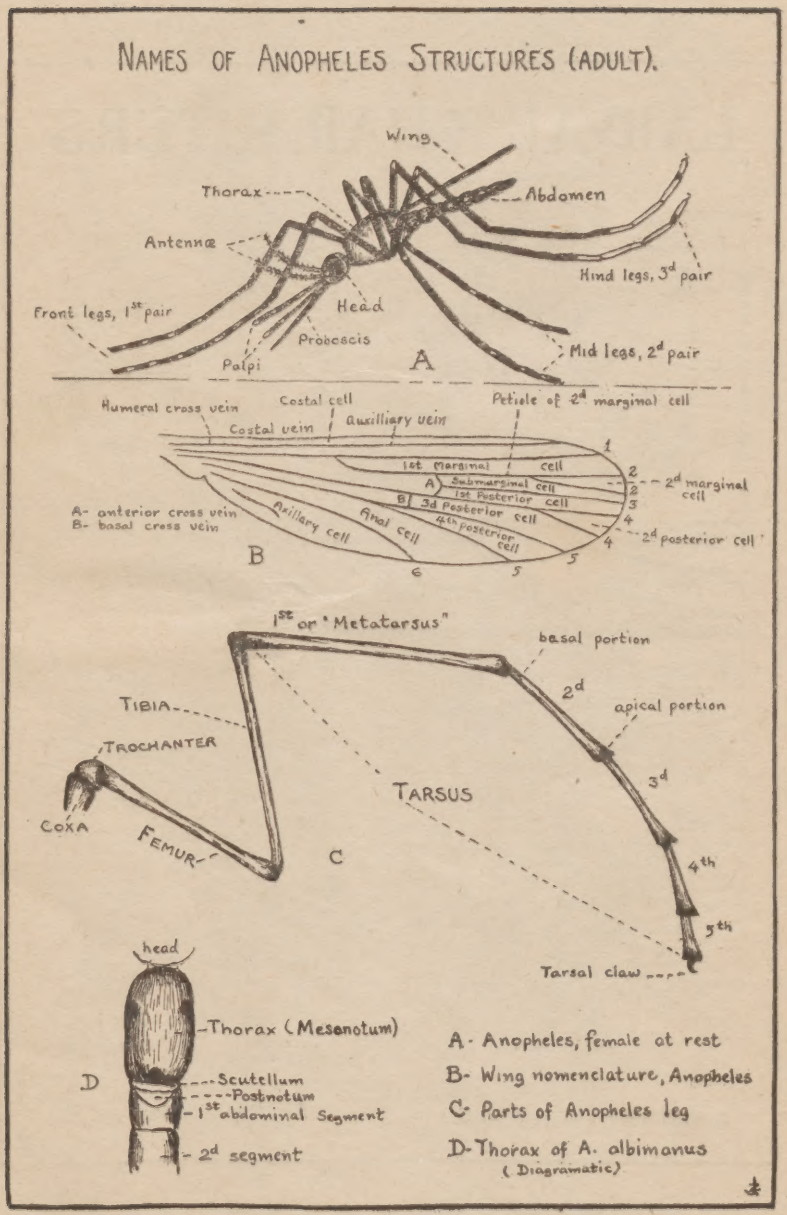

LARVAL CHARACTERS
mouth brushes
palpus
antenna
eye
HEAD
THORAX
ABDOMEN
1
2
3
4
5
6
7
8
palmate tufts
Ventral Brush
Position of lateral plates
dorsal plate on anal segment
Gills
A. ALBIMANUS (Diagrammatic)
albimanus & tarsimaculata
argyritarsis
pseudopunctipennis
eiseni
punctimacula
neivai
PALMATE TUFTS

PLATE F.


NAMES OF ANOPHELES STRUCTURES (ADULT).
Wing
Thorax
Abdomen
Antennæ
Hind legs, 3d pair
Front legs, 1st pair
Head
Proboscis
Palpi
A
Mid legs, 2d pair
Humeral cross vein
Costal cell
Petiole of 2d marginal cell
Costal vein
Auxilliary vein
1st Marginal cell
A Submarginal cell
2d marginal cell
1st Posterior cell
B 3d Posterior cell
A- anterior cross vein
B- basal cross vein
Axillary cell
Anal cell
4th posterior cell
2d posterior cell
B
1st or "Metatarsus"
basal portion
2d
apical portion
TIBIA
TROCHANTER
TARSUS
3d
COXA
FEMUR
C
4th
5th
Tarsal claw
head
Thorax (Mesonotum)
D
Scutellum
Postnotum
1st abdominal Segment
2d segment
A- Anopheles, female at rest
B- Wing nomenclature, Anopheles
C- Parts of Anopheles leg
D- Thorax of A. albimanus
(Diagramatic)